Association of *MDM2* SNP309, Age of Onset, and Gender in Cutaneous Melanoma

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Abstract

Purpose: In certain cancers, *MDM2* SNP309 has been associated with early tumor onset in women. In melanoma, incidence rates are higher in women than in men among individuals less than 40 years of age, but among those older than 50 years of age, melanoma is more frequent in men than in women. To investigate this difference, we examined the association among *MDM2* SNP309, age at diagnosis, and gender among melanoma patients.

Experimental Design: Prospectively enrolled melanoma patients ($N = 227$) were evaluated for *MDM2* SNP309 and the related polymorphism, p53 Arg72Pro. DNA was isolated from patient blood samples, and genotypes were analyzed by PCR-restriction fragment length polymorphism. Associations among *MDM2* SNP309, p53 Arg72Pro, age at diagnosis, and clinicopathologic features of melanoma were analyzed.

Results: The median age at diagnosis was 13 years earlier among women with a SNP309 GG genotype (46 years) compared with women with TG+TT genotypes (59 years; $P = 0.19$). Analyses using age dichotomized at each decade indicated that women with a GG genotype had significantly higher risks of being diagnosed with melanoma at ages <50 years compared with women ≥50 years, but not when the comparison was made between women <60 and ≥60 years. At ages <50 years, women with a GG genotype had a 3.89 times greater chance of being diagnosed compared with women with TG+TT genotypes ($P = 0.01$). Similar observations were not seen among men.

Conclusions: Our data suggest that *MDM2* may play an important role in the development of melanoma in women. The *MDM2* SNP309 genotype may help identify women at risk of developing melanoma at a young age.

Melanoma incidence rates vary dramatically with gender and age. According to National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) data (1), melanoma incidence rates are greater among women than men between the ages of 20 and 40 years, and among women in this age group melanoma incidence rates rise steeply. After the age of 40 years, melanoma incidence rates continue to rise in women, but at a slower pace. In men, the most rapid increase in incidence rates occurs between the ages of 50 and 80 years. As a result of these trends, the incidence rates of melanoma are greater among women than men at ages <40 years, equal in both sexes between the ages of 40 and 44 years, and greater among men than women at ages >45 years. Interestingly, the natural incidence of menopause prior to 40 years of age is quite low; however, nearly 10% of postmenopausal women in the United States are between the ages of 40 and 50 years, with many others entering the menopause transition or perimenopause during this decade (2). The average age of menopause in the United States and other Western countries is 51 years (3). Given these observations, it is possible that the discordance in melanoma incidence rates between men and women, both above and below the age of 50 years, is related in part to estrogen signaling.

Recently, a single nucleotide polymorphism (SNP) at position 309 in the P2 promoter of *MDM2* (rs2279744; T/G) has been associated with the onset of several different cancers among younger women. For example, women with an *MDM2* SNP309 *G* allele display earlier-onset soft tissue sarcoma, diffuse large B-cell lymphoma, colorectal cancer, and non–small cell lung cancer compared with patients lacking the *G* allele (4–10). In these studies, the differences in tumor onset were observed when patients were divided into "premenopausal" and "postmenopausal" groups using the age of 51 years as

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Translational Relevance

One of the principles of cancer medicine is early detection of localized disease to prevent metastatic disease. Ideally, early detection efforts are best applied to those patients at highest risk of a given cancer. This article describes a statistically significant relationship between age-related melanoma risk in women and an inherited genetic polymorphism. Although these results need to be replicated, the risk estimate is substantial for women <50 years of age, but not for older women, suggesting a role of female hormones in melanoma pathogenesis. On the clinical side, these findings may lead to a genetic test to identify women <50 years of age who are at increased risk of melanoma. These patients may benefit from more careful early detection screening. In the laboratory, these results may stimulate research into the role of female hormones in melanoma.

an approximation for menopausal status. Although these tumor types are not classically considered to be related to hormonal signaling, additional *in-vitro* studies showed a mechanistic link to estrogen signaling. The estrogen receptor serves as a cotranscriptional activator of the transcription factor Sp1 by binding to its COOH-terminal domain (11). The presence of the G nucleotide at SNP309 increases the binding affinity of Sp1 for the promoter, and transcriptional activity of the *MDM2* gene (12, 13). Studies of additional tumor types support the estrogen signaling hypothesis. For example, among ovarian cancers and invasive ductal carcinomas of the breast, the association between age of onset and the SNP309 genotype was only observed among estrogen receptor–positive (versus estrogen receptor–negative) tumors (4, 14). More recently, endometrial cancer risk was found to be greater in women with a SNP309 GG genotype as compared with women with other genotypes, an important observation given that endometrial cancer risk increases with unopposed estrogen stimulation of the uterus (15).

MDM2 is a key negative regulator of the tumor suppressor p53. Via its E3 ubiquitin ligase properties, MDM2 targets p53 for proteasomal degradation (16–18). In a subset of human tumors, overexpression of MDM2 is associated with accelerated cancer progression and lack of response to therapy (19). These observations suggest that MDM2 overexpression may substitute for p53 mutations in these tumors. Melanoma belongs to a group of tumors for which p53 mutations are rare. Unexpectedly, our group found that MDM2 overexpression is an independent predictor of improved survival in melanoma (20), a finding that was later reproduced by another group of investigators (21). Similar observations have been made in other tumor types. MDM2 accumulation correlates with favorable clinical-pathologic parameters in patients with esophageal, ovarian, colon, and non–small cell lung cancer (22–25), suggesting that MDM2 accumulation may not drive an aggressive, malignant phenotype in all tumors. A recent study in ovarian carcinoma found that the *MDM2* SNP309 G allele correlated with increased overall survival despite an earlier age of onset (14).

Several studies in other tumors have examined the association between *MDM2* SNP309 and a well-studied polymorphism in p53, Arg72Pro (rs1042522; R/P). The results have shown positive associations between the proline/proline (PP) genotype and disease risk for some tumor types (e.g. esophageal squamous cell carcinoma, lung cancer, renal cell carcinoma) but no association for other tumor types (e.g. colorectal cancer, breast cancer; refs. 26–30). The association between the p53 Arg72Pro polymorphism itself and melanoma risk is controversial (31–34), with some studies showing associations between melanoma risk and the PP genotype (31–33), but others showing increased melanoma risk with the arginine/arginine (RR) genotype (34).

Our pilot study examines the relationship among *MDM2* SNP309, p53 Arg72Pro, and patient and tumor clinicopathologic factors in a population of newly diagnosed melanoma patients.

Materials and Methods

Patient population. The study cohort consisted of 227 newly diagnosed primary melanoma patients prospectively enrolled in the Interdisciplinary Melanoma Cooperative Group at the New York University School of Medicine from August 2002 to November 2006. Clinicopathologic, demographic, and survival data were recorded prospectively for all patients. The New York University Institutional Review Board approved this study and informed consent was obtained from all patients at the time of enrollment.

Genotype analysis. Genomic DNA was isolated from 227 peripheral blood leukocyte specimens collected at the time of patient enrollment (AutoGen, QuickGene Mini80). Twenty to 100 ng of genomic DNA from each sample were amplified by PCR using published primers and conditions for *MDM2* SNP309 (12) and p53 Arg72Pro (34). Genotypes were determined by restriction fragment length polymorphism analysis using *MspA1I* (New England Biolabs) for SNP309, and *BstUI* and *BtgI* (NEB) for Arg72Pro. Genotype assays contained negative and positive control DNAs. Eleven melanoma cell lines (SKMEL 19, 29, 85, 94, 100, 103, 147, 173, 187, 192, and 197) were also analyzed for *MDM2* SNP309 and p53 Arg72Pro genotypes.

Statistical methods. The characteristics of melanoma patients were summarized using medians with interquartile ranges (IQR) for continuous variables and percentages for categorical variables. Wilcoxon rank sum tests (for continuous variables) and χ^2 statistics and Fisher's exact tests (for categorical variables) were used to identify differences between men and women.

The associations between the SNP309 and Arg72Pro genotypes were evaluated using Fisher's exact tests. Histopathologic features of melanoma were compared by genotype separately for men and women using Kruskal-Wallis tests for continuous variables and χ^2 statistics for categorical variables. The distributions of age of diagnosis by SNP309 genotype and Arg72Pro genotype are presented graphically for men and women using boxplots. The odds of being diagnosed with melanoma by a specified age for patients with the SNP309 GG genotype were compared to the odds of diagnosis by this age for patients with SNP309 TG or TT genotypes. This was done using odds ratios with 95% confidence intervals. These odds ratios were estimated for selected cutpoints of the age distribution.

Statistical analyses were done at a significance level of 0.05 (two-sided). No adjustments were made for multiple comparisons. SAS 9.1 and SPSS 14.0 were used for statistical analyses.

Results

Patient characteristics. Table 1 presents a summary of patient and tumor characteristics. A total of 227 primary

Table 1. Characteristics of study population

A. Patient Characteristics	**Total**	**Women**	**Men**
	***n*(%)**	***n* (%)**	***n* (%)**
All melanoma patients	227 (100)	93 (41.0)	134 (59.0)
Median age at diagnosis (y)	58	57	58.5
History of multiple melanoma	29 (12.8)	12 (12.9)	17 (12.7)
Family history of melanoma	23 (10.1)	11 (11.8)	12 (9.0)
Stage of disease at recruitment			
I	171 (75.3)	74 (79.6)	97 (72.4)
II	38 (16.7)	14 (15.1)	24 (17.9)
III	18 (7.9)	5 (5.4)	13 (9.7)
B. Tumor Characteristics	**Total**	**Women**	**Men**
	***n* (%)**	***n* (%)**	***n* (%)**
Histologic type			
Superficial spreading	111 (48.9)	46 (49.5)	65 (48.5)
Nodular	49 (21.6)	16 (17.2)	33 (24.6)
Other*	32 (14.1)	16 (17.2)	16 (11.9)
Unclassified	35 (15.4)	15 (16.1)	20 (14.9)
Median thickness (mm)	0.85	0.72	0.9
Anatomic site			
Axial	132 (58.1)	38 (40.9)	94 (70.1)
Extremity	95 (41.9)	55 (59.1)	40 (29.9)
Ulceration	34 (15.0)	12 (12.9)	22 (16.4)
Recurrence	14 (6.2)	5 (5.4)	9 (6.7)

NOTE: Unknown values are not included in the table.
*"Other" comprises desmoplastic, lentigo maligna and acral lentiginous, as well as those melanomas that did not fit into one histologic category.

melanoma patients were studied. Ninety-eight percent of patients were Caucasian. The gender distribution of these patients (59% men and 41% women) is nearly identical to the gender distribution of melanoma patients in the United States (1). The median age at melanoma diagnosis was 58 years, similar to the national median of 59 years. The distribution of melanomas by stage was 75.3% stage I, 16.7% stage II, 7.9% stage III, and 0% stage IV. No differences were found between men and women with respect to personal or family history of melanoma, histologic type, median tumor thickness, ulceration, or recurrence. However, the anatomic site of melanoma did differ significantly between men and women ($P < 0.001$, Fisher's exact test). This observation is in accordance with published epidemiologic data (35). In addition, 98% of patients in the study were Caucasian, as is typical for the incidence of melanoma in the U.S. population.

MDM2 SNP309 and p53 Arg72Pro genotypes in patient germline DNA. As an initial analysis, we compared the PCR-restriction fragment length polymorphism genotyping methodology with routine sequencing of DNA extracted from 11 melanoma cell lines available in the lab. We found complete concordance between these methods, and used the PCR-restriction fragment length polymorphism method for the patient samples. An example of the genotyping results is shown in Fig. 1. Among the cell lines, the distribution of *MDM2* SNP309 genotypes was TT 18.2%, TG 54.5%, and GG 27.3%. The distribution of p53 Arg72Pro genotypes was RR 72.7%, RP 0%, and PP 27.3%. Supplemental Table S1 shows the SNP309 and Arg72Pro genotypes for each melanoma cell line. We subsequently examined *MDM2* SNP309 and p53 Arg72Pro genotypes in the cohort of melanoma patients described above. We successfully amplified DNA from 216 of 227 (95.2%) patients for *MDM2* SNP309, and from 213 of 227 (93.8%) patients for p53 Arg72Pro. *MDM2* SNP309 genotype frequencies were TT 31.5%, TG 44.4%, and GG 24.1%; p53 Arg72Pro genotype frequencies were RR 57.7%, RP 35.2%, and PP 7.0%.

Analysis of SNP309 and p53 Arg72Pro genotypes and clinicopathologic variables. We analyzed the histopathologic features of each patient's tumor specimen for association with the MDM2 and p53 genotypes. There were no associations between the polymorphisms and histopathologic subtype, tumor thickness, anatomic site, and tumor ulceration (Table 2A and B). In addition we did not find any association between these genetic polymorphisms and patient recurrence or overall survival (data not shown).

Association between SNP309 genotype, patient gender, and age at melanoma diagnosis. Figure 2 presents boxplots that summarize the distribution of age at diagnosis for *MDM2* SNP309 and p53 Arg72Pro genotypes by gender. Overall, the median age at diagnosis in men was 58.5 years compared with

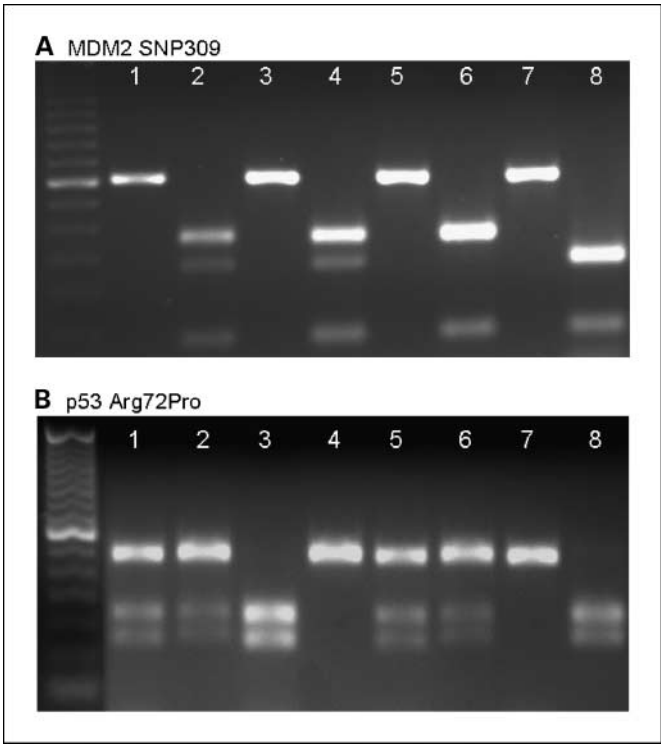

Fig. 1. PCR-restriction fragment length polymorphism analysis of SNP309 and Arg72Pro genotypes. Each pair of lanes (e.g., 1+2, 3+4, etc.) represents the analysis from a single patient. A 50-bp ladder (*leftmost lane*) was used to distinguish band size. *A,* MDM2 SNP309. Odd lanes were loaded with PCR products, and even lanes with the products of an *MspA1I* digest. One upper band (233 bp) represents the TT genotype (*lane 6*), two bands (233 and 187 bp) represent the TG genotype (*lanes 2 and 4*), and one lower band (187 bp) represents the GG genotype (*lane 8*). *B,* p53 Arg72Pro. Odd lanes were loaded with the products of the *BstUI* digest, even lanes with the products of the *BtgI* digest. In odd lanes, one upper band (296 bp) represents the PP genotype (*lane 7*), three bands (296, 169, and 127 bp) represent the RP genotype (*lanes 1 and 5*), and two lower bands (169, 127 bp) represent the RR genotype (*lane 3*). In even lanes, one upper band (296 bp) represents the RR genotype (*lane 4*), three bands (296, 169, and 127 bp) represent the RP genotype (*lanes 2 and 6*), and two lower bands (169, 127 bp) represent the PP genotype (*lane 8*).

Table 2.

A. Histopathologic features of melanoma by MDM2 SNP309 genotype

Patient and tumor characteristics	Women (*n* = 89)				Men (*n* = 127)			
	TT	TG	GG	*P*	TT	TG	GG	*P*
	n (%)	*n* (%)	*n* (%)		(%)	*n* (%)	*n* (%)	
All Melanoma Patients	33 (37.1)	35 (39.3)	21 (23.6)	—	35 (27.6)	61 (48.0)	31 (24.4)	—
History of multiple melanoma	4 (12.5)	5 (14.7)	1 (5.0)	0.63	4 (11.8)	9 (15.8)	3 (10.0)	0.78
Family history of melanoma	6 (20.7)	3 (9.1)	2 (10.5)	0.42	3 (8.8)	5 (9.3)	4 (14.8)	0.73
Stage of disease								
I	26 (78.8)	28 (80.0)	18 (85.7)	0.81	28 (80.0)	40 (65.6)	23 (74.2)	0.56
II	4 (12.1)	5 (14.3)	3 (14.3)	—	5 (14.3)	14 (23.0)	4 (12.9)	—
III	3 (9.1)	2 (5.7)	0 (0)	—	2 (5.7)	7 (11.5)	4 (12.9)	—
Histologic type								
SS	15 (45.5)	17 (48.6)	12 (57.1)	0.72	17 (48.6)	26 (42.6)	15 (48.4)	0.8
Nodular	4 (12.1)	8 (22.9)	4 (19.0)	—	7 (20.0)	20 (32.8)	6 (19.4)	—
Other*	6 (18.2)	6 (17.1)	3 (14.3)	—	5 (14.3)	7 (11.5)	4 (12.9)	—
Unclassified	8 (24.2)	4 (11.4)	2 (9.5)	—	6 (17.1)	8 (13.1)	6 (19.4)	—
Tumor thickness, mm (median, IQR)	0.53 (1.05)	0.85 (1.05)	0.8 (0.92)	0.41 †	0.78 (0.87)	1.12 (2.0)	0.90 (1.15)	0.35 †
Anatomic site								
Axial	13 (39.4)	13 (37.1)	10 (47.6)	0.62	24 (68.6)	45 (73.8)	22 (71.0)	0.87
Extremity	20 (60.6)	22 (62.9)	11 (52.4)	—	11 (31.4)	16 (26.2)	9 (29.0)	—
Ulceration	2 (6.1)	8 (22.9)	2 (9.5)	0.12	4 (11.4)	14 (23.0)	3 (9.7)	0.2
Recurrence	3 (9.1)	2 (5.7)	0 (0)	0.36	1 (2.9)	6 (9.8)	2 (6.5)	0.5

B. Histopathologic features of melanoma by p53 Arg72Pro genotype

Patient and tumor characteristics	Women (*n* = 87)				Men (*n* = 126)			
	RR	RP	PP	*P*	RR	RP	PP	*P*
	n (%)	*n* (%)	*n* (%)		n (%)	*n* (%)	*n* (%)	
All melanoma patients	52 (59.8)	29 (33.3)	6 (6.9)	—	71 (56.3)	46 (36.5)	9 (7.1)	—
History of multiple melanoma	7 (13.5)	3 (10.3)	0 (0.0)	1	6(9.0)	9 (20.0)	1 (11.1)	0.27
Family history of melanoma	8 (17.0)	1 (3.4)	1 (20.0)	0.18	6 (8.5)	5 (10.9)	1 (11.1)	1
Stage of disease								
I	43 (82.7)	22 (75.9)	4 (66.7)	0.53	54 (76.1)	31 (67.4)	5 (55.6)	0.47
II	7 (13.5)	5 (17.2)	1 (16.7)	—	11 (15.5)	10 (21.7)	2 (22.2)	—
III	2 (3.8)	2 (6.9)	1 (16.7)	—	6 (8.5)	5 (10.9)	2 (22.2)	—
Histologic type								
SS	28 (53.8)	14 (48.3)	2 (33.3)	0.81	32 (45.1)	21 (45.7)	4 (44.4)	0.89
Nodular	7 (13.5)	6 (20.7)	2 (33.3)	—	16 (22.5)	14 (30.4)	3 (33.3)	—
Other*	9 (17.3)	6 (20.7)	1 (16.7)	—	9 (12.7)	6 (13.0)	1 (11.1)	—
Unclassified	8 (15.4)	3 (10.3)	1 (16.7)	—	14 (19.7)	5 (10.9)	1 (11.1)	—
Tumor thickness, mm (median, IQR)	0.70 (0.91)	0.77 (1.05)	0.88 (8.04)	0.83 †	0.76 (1.15)	0.90 (1.48)	1.70 (2.33)	0.07 †
Anatomic site								
Axial	20 (38.5)	13 (44.8)	2 (33.3)	0.78	52 (73.2)	33 (71.7)	6 (66.7)	0.86
Extremity	32 (61.5)	16 (55.2)	4 (66.7)	—	19 (26.8)	13 (28.3)	3 (33.3)	—
Ulceration	6 (11.5)	4 (13.8)	2 (33.3)	0.26	9 (12.7)	9 (19.6)	3 (33.3)	0.2
Recurrence	2 (3.8)	2 (6.9)	1 (16.7)	0.31	4 (5.6)	4 (8.7)	1 (11.1)	0.63

NOTE: *P* value calculated by Fisher's exact test unless otherwise specified.
Unknown values are not included in the table. Stage of disease was at time of recruitment.
Abbreviation: SS, superficial spreading.
*"Other" comprises desmoplastic, lentigo maligna, and acral lentiginous, as well as those melanomas that did not fit into one histologic category.
† Kruskal-Wallis test.

57 years in women. Among women, age at diagnosis ranged from 19 to 97 years of age, and was 13 years earlier among women with the *MDM2* SNP309 GG genotype (median, 46 years; IQR, 31) as compared with women with TG+TT genotypes (median, 59 years; IQR, 19; $P = 0.19$, Mann-Whitney). Among men, age at diagnosis ranged from 25 to 92 years of age, and was approximately equal between men with the *MDM2* SNP309 GG genotype (median, 60 years; IQR, 21.5) and men with TG+TT genotypes (median, 58 years; IQR, 19.5; $P = 0.48$, Mann-Whitney).

The distributions of *MDM2* SNP309 genotypes among patients in the 10-year age-at-diagnosis groups are shown in Table 3. Comparing women with the GG genotype to women with either TT or TG phenotypes, we observed that by the age of 50 years, 11 of 21 (52.4%) of women with a GG genotype were diagnosed with melanoma, compared with 15 of 68 (22.1%) of

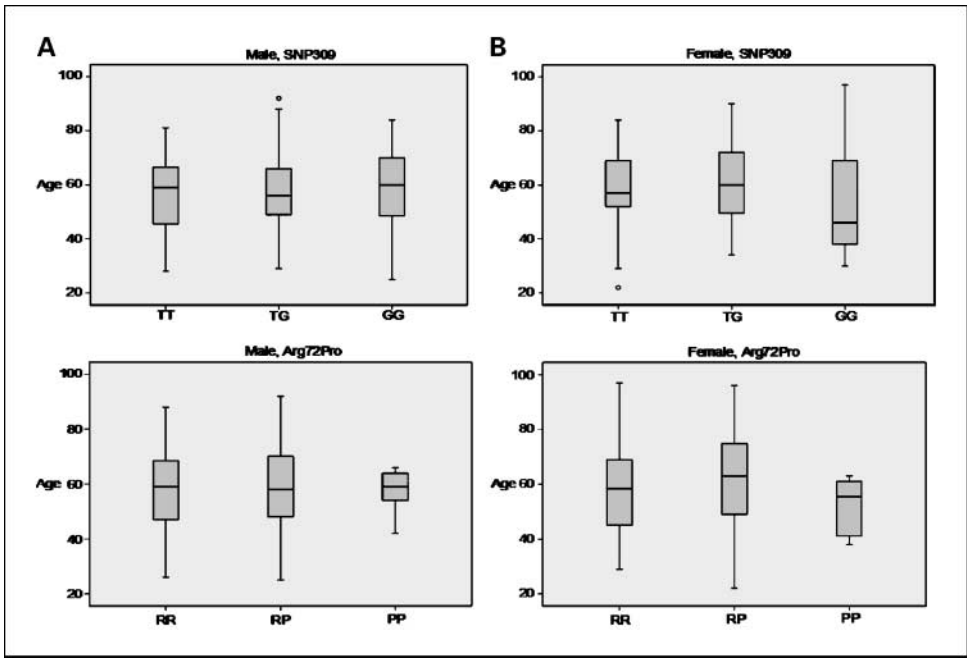

Fig. 2. Age at melanoma diagnosis by SNP309 and Arg72Pro genotypes for men and women. These boxplots display the distributions of age at diagnosis for patients categorized by gender and genotype. Each box marks off the lower and upper quartile of an age distribution; the line within the box represents the median age. The vertical lines outside the box terminate at the largest and smallest values beyond the box that are within 1.5 times the IQR from the box; any point beyond 1.5 IQRs from the box is considered an outlier, as is indicated by the circles in (*A*) and (*B*). The relatively low age at diagnosis for women with the SNP309 GG genotype is evident in *B*.

women with either a TT or TG genotype (P = 0.0126, Fisher's exact test). Among women with the GG genotype, the highest proportion, 8 of 21 (38.1%), were diagnosed between the ages of 30 and 39 years. Among women with TG and TT genotypes, only 6 of 68 (8.8%) were diagnosed in the 30- to 39-year-old age group (P = 0.0034, Fisher's exact test). These differences were not observed in men.

Table 4 shows the odds ratios for initial diagnosis of melanoma below a specified age (with age dichotomized at 10-year intervals) for patients with the SNP309 GG genotype as compared with patients with SNP309 TG+TT genotypes. This is shown separately for men and women. At age cutpoints below 50 years of age, women with the SNP309 GG genotype are more likely to be diagnosed with melanoma in the younger age category as compared with women with TG+TT genotypes; at age cutpoints of ≥60 years, the association is weak. Women with the *MDM2* SNP309 GG genotype had a 3.89 times greater chance of being diagnosed at <50 years of age as compared with women with TG+TT genotypes (95% confidence interval, 1.22-12.31; P = 0.01). Note that the odds ratio was 4.62 (95% confidence interval, 1.23-16.86; P = 0.02) when an age of <40 years was used as a cut point, although there was considerable overlap between the confidence intervals for the odds ratios when age was cut at 40 and at 50 years. These associations were not seen among men. p53 Arg72Pro genotypes were not associated with melanoma risk in women or men.

Discussion

Our study identified an important association among age at melanoma diagnosis, female gender, and the GG genotype for *MDM2* SNP309. Although overall *MDM2* SNP309 genotype frequencies did not differ between genders, women diagnosed at younger ages were more likely to have an *MDM2* SNP309 GG genotype as compared with older women. The greatest odds ratio for the diagnosis of melanoma among women with a SNP309 GG genotype was for those <40 years of age and the association remained significant as age increased to 50 years. The association was weak for ≥50 years of age. The decrease in the odds ratio from 4.62 to 3.89 as the age cutpoint increased from age 40 to age 50 years may reflect the fact that it is not uncommon for women to undergo menopause prior to the age of 50, but it is rare for this to occur prior to the age of 40 years (2, 3). These findings, combined with the SEER epidemiologic observation that prior to the age of 40 years melanoma is more common among women than men (but not after the age of 50 years), support the hypothesis that active estrogen signaling in combination with the GG genotype may contribute to melanoma onset in women. Nevertheless, this pilot study was limited by not having information regarding the menopausal status of our patients at the time of their melanoma diagnosis. A follow-up replication study will include these data to more precisely characterize the association between the SNP309 genotype and menopausal status at the time of melanoma diagnosis.

The role of estrogen in the development of melanoma remains controversial. The debate began in the 1970s when two initial observations were made: (*a*) young women taking oral contraceptives seemed to be at increased risk for melanoma (36), and (*b*) pregnancy-related skin pigmentation changes were linked to the accelerated synthesis of melanin by estrogen (37). Although some follow-up studies found associations among oral contraceptives, hormonal replacement, pregnancy, and melanoma (38–41), others did not (42–46). In addition, immunohistochemical studies in melanoma showed little or no staining for what is now recognized as estrogen receptor α (47–50), but type-II, low-affinity estrogen binding sites were detected in melanoma (51). Interestingly, estrogen receptor β, a subsequently discovered isoform of the estrogen receptor (52), was recently found to be expressed in 100% of 94 melanocytic lesions, whereas only 7.5% of these lesions expressed estrogen

Table 3. MDM2 SNP309 genotype distribution in 10-year age-at-diagnosis groups

Women

Age at diagnosis (y)	TT+TG	GG	Total
	n (%)	*n* (%)	*n* (%)
19-29	2 (2.9)	0 (0)	2 (2.2)
30-39	6 (8.8)	8 (38.1)	14 (15.7)
40-49	7 (10.3)	3 (14.3)	10 (11.2)
50-59	19 (27.9)	1 (4.8)	20 (22.5)
60-69	16 (23.5)	4 (19.0)	20 (22.5)
70-79	11 (16.2)	3 (14.3)	14 (15.7)
80-89	6 (8.8)	0 (0)	6 (6.7)
90-99	1 (1.5)	2 (9.5)	3 (3.4)
Total	68 (100)	21 (100)	89 (100)

$P = 0.0098$ (χ^2)

Men

Age at diagnosis (y)	TT+TG	GG	Total
	n (%)	*n* (%)	*n* (%)
19-29	2 (2.1)	4 (12.9)	6 (4.7)
30-39	6 (6.3)	2 (6.5)	8 (6.3)
40-49	19 (19.8)	2 (6.5)	21 (16.5)
50-59	26 (27.1)	7 (22.6)	33 (26.0)
60-69	26 (27.1)	7 (22.6)	33 (26.0)
70-79	14 (14.6)	7 (22.6)	21 (16.5)
80-89	2 (2.1)	2 (6.5)	4 (3.1)
90-99	1 (1.0)	0 (0)	1 (0.8)
Total	96 (100)	31 (100)	127 (100)

$P = 0.12$ (χ^2)

NOTE: Percentages displayed are column percentages, representing percents within genotypes.

receptor α (53). A meta-analysis of clinical trials using tamoxifen, a selective estrogen receptor modulator, in patients with metastatic melanoma showed that tamoxifen did not improve response or survival when administered with combined chemotherapy regimens (54); however, recent breast cancer studies have shown that estrogen receptor β may affect tumor resistance to tamoxifen (55–57), so it is conceivable that the failure of tamoxifen to improve outcomes in melanoma patients is related to the greater expression of estrogen receptor β versus estrogen receptor α in melanoma.

Limitations of the current study include the lack of a group of patients unaffected by melanoma, and lack of patient ancestry data. Regarding nonmelanoma controls, *MDM2* SNP309 genotypes have been analyzed in over 2,000 control subjects worldwide (4, 6–8, 12, 26, 28, 58–63). The GG genotype frequency in these control individuals ranged from 10% to 34%, with no substantial variation between men and women. Variation in allele frequencies has been observed among ethnic populations and could potentially bias our results if the patients from one ethnic group were overrepresented in a particular grouping of patients being analyzed. For example, Caucasians of Ashkenazi Jewish descent have been noted to harbor a higher frequency of GG genotypes (64); however, the analysis of a subset of our patients ($n = 45$) for whom self-reported ancestry data were available did not reveal an enrichment of Ashkenazi female patients <50 years of age, suggesting that it is unlikely that our results are biased by enrichment of patients from that ethnic group.

Recently, an analysis of *MDM2* SNP309, p53 Arg72Pro, and skin cancer risk was published using patients from the Nurses Health Study. Among the 219 melanoma patients studied, no significant associations were observed between either of these polymorphisms and the risk of melanoma. There were also no statistically significant associations between these polymorphisms and the risk of basal cell carcinoma or squamous cell carcinoma (65). As they did not present an estimate of the risk of melanoma by age group, it is difficult to directly compare their results with ours. However, an important difference between these cohorts was the frequency of the GG genotype. Among their patients, the frequency of the GG genotype was 13% versus 24% in our cohort. Thus, they may have been unable to detect the association that we describe due to a lack of sufficient numbers of patients with the GG genotype. This difference in the frequencies of the GG genotype between the two cohorts may be related to differences in ancestry among the patients enrolled. Alternatively, it may be related to patient age at diagnosis. We found a high GG frequency (42%) in women diagnosed at <50 years of age, and a much lower frequency (14%) in women diagnosed at ≥50 years of age. The mean age of melanoma diagnoses in their cohort was 63.4 years versus 57 years for both the mean and median of women in our cohort. Therefore, it is possible that the lower GG genotype frequency in their cohort is related to the higher mean age of their patients compared with our patients.

Studies in other cancers have reported a combined effect of both *MDM2* and *p53* polymorphisms on cancer risk and survival (26–28). The effect of p53 Arg72Pro on melanoma remains controversial, with some studies reporting associations between melanoma and the PP genotype (31–33) and others reporting associations between melanoma and the RR genotype (34). In our study, the distribution of p53 Arg72Pro genotypes did not vary by age or gender, and there was no evidence suggesting a strong association between the p53 Arg72Pro genotypes and melanoma risk, including a possible combined effect of both *MDM2* SNP309 and p53 Arg72Pro genotypes on melanoma risk.

Several studies have examined the effect of *MDM2* SNP309 on cancer survival. Studies on gastric and renal cell carcinomas have shown that *MDM2* SNP309 associates with decreased

Table 4. Odds ratios and exact 95% confidence intervals for initial diagnosis of melanoma at ages less than the age shown below, for the MDM2 SNP309 GG genotype compared to other MDM2 SNP309 genotypes

Age cutpoint (y)	Women (*n* = 89)	Men (*n* = 127)
	OR (95% CI)	OR (95% CI)
<40 vs ≥40	4.62 (1.23, 16.86)	2.64 (0.68, 9.55)
<50 vs ≥50	3.89 (1.22, 12.31)	0.89 (0.31, 2.39)
<60 vs ≥60	1.33 (0.44, 4.08)	0.76 (0.31, 1.86)
<70 vs ≥70	1.15 (0.34, 4.60)	0.53 (0.19, 1.54)
<80 vs ≥80	1.09 (0.19, 11.61)	0.47 (0.05, 5.89)

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

survival (28, 60); however, a recent study on ovarian cancer has shown that *MDM2* SNP309 associates with improved survival (14). Data from our group and others have shown that accumulation of MDM2 protein in primary melanoma is an independent predictor of improved survival (20, 21). It is possible that the increased expression of MDM2 in these patients was due to the presence of a SNP309 GG genotype. Unfortunately, germline DNA from the patients in our previous study is not available for analysis. In the current study, mean follow-up time was not sufficient to analyze the effects of *MDM2* SNP309 on survival. Future studies will be conducted to address this question.

In conclusion, our pilot study suggests that *MDM2* may play an important role in the development of melanoma in women. The *MDM2* SNP309 genotype may help identify women at risk of developing melanoma at a young age. Also, these data suggest that it may be worthwhile to revisit the effects of estrogen on melanoma in the context of the appropriate *MDM2* SNP309 genotype.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Ries L, Melbert D, Krapcho M, et al. SEER Cancer Statistics Review, 1975–2005, National Cancer Institute. Bethesda (MD). Based on November 2007 SEER data submission, posted to the SEER web site, 2008.
2. NAMS. Overview of menopause and aging. Menopause practice: a clinician's guide. 3rd ed. Cleveland: The North American Menopause Society Professional Education Committee; 2007. p. 9–17.
3. Nelson HD. Menopause. Lancet 2008;371:760–70.
4. Bond GL, Hirshfield KM, Kirchhoff T, et al. MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. Cancer Res 2006;66:5104–10.
5. Bond GL, Menin C, Bertorelle R, Alhopuro P, Aaltonen LA, Levine AJ. MDM2 SNP309 accelerates colorectal tumour formation in women. J Med Genet 2006;43:950–2.
6. Lind H, Zienolddiny S, Ekstrom PO, Skaug V, Haugen A. Association of a functional polymorphism in the promoter of the MDM2 gene with risk of nonsmall cell lung cancer. Int J Cancer 2006;119:718–21.
7. Menin C, Scaini MC, De Salvo GL, et al. Association between MDM2-SNP 309 and age at colorectal cancer diagnosis according to p53 mutation status. J Natl Cancer Inst 2006;98:285–8.
8. Alhopuro P, Ylisaukko-Oja SK, Koskinen WJ, et al. The MDM2 promoter polymorphism SNP309T->G and the risk of uterine leiomyosarcoma, colorectal cancer, and squamous cell carcinoma of the head and neck. J Med Genet 2005;42:694–8.
9. Yarden RI, Friedman E, Metsuyanim S, Olender T, Ben-Asher E, Papa MZ. MDM2 SNP309 accelerates breast and ovarian carcinogenesis in BRCA1 and BRCA2 carriers of Jewish-Ashkenazi descent. Breast Cancer Res Treat 2008;111:497–504. Epub 2007 Nov 18.
10. Atwal GS, Rabadan R, Lozano G, et al. An information-theoretic analysis of genetics, gender and age in cancer patients. PLoS ONE 2008;3:e1951.
11. Saville B, Wormke M, Wang F, et al. Ligand-, cell-, and estrogen receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements. J Biol Chem 2000;275:5379–87.
12. Bond GL, Hu W, Bond EE, et al. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 2004;119:591–602.
13. Hu W, Feng Z, Ma L, et al. A single nucleotide polymorphism in the MDM2 gene disrupts the oscillation of p53 and MDM2 levels in cells. Cancer Res 2007; 67:2757–65.
14. Bartel F, Jung J, Bohnke A, et al. Both germ line and somatic genetics of the p53 pathway affect ovarian cancer incidence and survival. Clin Cancer Res 2008; 14:89–96.
15. Grady D, Gebretsadik T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol 1995;85:304–13.
16. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature 1997; 387:296–9.
17. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. Nature 1997;387:299–303.
18. Bond GL, Hu W, Levine AJ. MDM2 is a central node in the p53 pathway: 12 years and counting. Curr Cancer Drug Targets 2005;5:3–8.
19. Freedman DA, Levine AJ. Regulation of the p53 protein by the MDM2 oncoprotein-thirty-eighth G H.A. Clowes Memorial Award Lecture. Cancer Res 1999;59:1–7.
20. Polsky D, Melzer K, Hazan C, et al. HDM2 protein overexpression and prognosis in primary malignant melanoma. J Natl Cancer Inst 2002;94:1803–6.
21. Berger AJ, Camp RL, Divito KA, Kluger HM, Halaban R, Rimm DL. Automated quantitative analysis of HDM2 expression in malignant melanoma shows association with early-stage disease and improved outcome. Cancer Res 2004;64:8767–72.
22. Abdel-Fattah G, Yoffe B, Krishnan B, Khaoustov V, Itani K. MDM2/p53 protein expression in the development of colorectal adenocarcinoma. J Gastrointest Surg 2000;4:109–14.
23. Baekelandt M, Kristensen GB, Nesland JM, Trope CG, Holm R. Clinical significance of apoptosis-related factors p53, Mdm2, and Bcl-2 in advanced ovarian cancer. J Clin Oncol 1999;17:2061.
24. Higashiyama M, Doi O, Kodama K, et al. MDM2 gene amplification and expression in non-small-cell lung cancer: immunohistochemical expression of its protein is a favourable prognostic marker in patients without p53 protein accumulation. Br J Cancer 1997; 75:1302–8.
25. Saito H, Tsujitani S, Oka S, Ikeguchi M, Maeta M, Kaibara N. The expression of murine double minute 2 is a favorable prognostic marker in esophageal squamous cell carcinoma without p53 protein accumulation. Ann Surg Oncol 2002;9:450–6.
26. Hong Y, Miao X, Zhang X, et al. The role of P53 and MDM2 polymorphisms in the risk of esophageal squamous cell carcinoma. Cancer Res 2005;65: 9582–7.
27. Zhang X, Miao X, Guo Y, et al. Genetic polymorphisms in cell cycle regulatory genes MDM2 and TP53 are associated with susceptibility to lung cancer. Hum Mutat 2006;27:110–7.
28. Hirata H, Hinoda Y, Kikuno N, et al. MDM2 SNP309 polymorphism as risk factor for susceptibility and poor prognosis in renal cell carcinoma. Clin Cancer Res 2007;13:4123–9.
29. Talseth BA, Meldrum C, Suchy J, Kurzawski G, Lubinski J, Scott RJ. MDM2 SNP309 T>G alone or in combination with the TP53 R72P polymorphism does not appear to influence disease expression and age of diagnosis of colorectal cancer in HNPCC patients. Int J Cancer 2007;120:563–5.
30. Schmidt MK, Reincke S, Broeks A, et al. Do MDM2 SNP309 and TP53 R72P interact in breast cancer susceptibility? A large pooled series from the breast cancer association consortium. Cancer Res 2007;67: 9584–90.
31. Stefanaki I, Stratigos AJ, Dimisianos G, et al. p53 codon 72 Pro homozygosity increases the risk of cutaneous melanoma in individuals with dark skin complexion and among noncarriers of melanocortin 1 receptor red hair variants. Br J Dermatol 2007;156: 357–62.
32. Han J, Cox DG, Colditz GA, Hunter DJ. The p53 codon 72 polymorphism, sunburns, and risk of skin cancer in US Caucasian women. Mol Carcinog 2006; 45:694–700.
33. Gwosdz C, Scheckenbach K, Lieven O, et al. Comprehensive analysis of the p53 status in mucosal and cutaneous melanomas. Int J Cancer 2006;118:577–82.
34. Shen H, Liu Z, Strom SS, et al. p53 codon 72 Arg homozygotes are associated with an increased risk of cutaneous melanoma. J Invest Dermatol 2003;121: 1510–4.
35. Kopf AW, Welkovich B, Frankel RE, et al. Thickness of malignant melanoma: global analysis of related factors. J Dermatol Surg Oncol 1987;13:345–90, 401–20.
36. Beral V, Ramcharan S, Faris R. Malignant melanoma and oral contraceptive use among women in California. Br J Cancer 1977;36:804–9.
37. Hall PF. The influence of hormones on melanogenesis. Australas J Dermatol 1969;10:125–39.
38. Ellis DL, Wheeland RG. Increased nevus estrogen and progesterone ligand binding related to oral contraceptives or pregnancy. J Am Acad Dermatol 1986;14:25–31.
39. Holly EA, Cress RD, Ahn DK. Cutaneous melanoma in women: ovulatory life, menopause, and use of exogenous estrogens. Cancer Epidemiol Biomarkers Prev 1994;3:661–8.
40. Holly EA, Weiss NS, Liff JM. Cutaneous melanoma in relation to exogenous hormones and reproductive factors. J Natl Cancer Inst 1983;70:827–31.
41. Feskanich D, Hunter DJ, Willett WC, et al. Oral contraceptive use and risk of melanoma in premenopausal women. Br J Cancer 1999;81:918–23.
42. Lederman JS, Lew RA, Koh HK, Sober AJ. Influence of estrogen administration on tumor characteristics and survival in women with cutaneous melanoma. J Natl Cancer Inst 1985;74:981–5.
43. Lea CS, Holly EA, Hartge P, et al. Reproductive risk factors for cutaneous melanoma in women: a case-control study. Am J Epidemiol 2007;165:505–13.
44. Osterlind A, Tucker MA, Stone BJ, Jensen OM. The

Danish case-control study of cutaneous malignant melanoma. III. Hormonal and reproductive factors in women. Int J Cancer 1988;42:821–4.

45. Silipo V, De Simone P, Mariani G, Buccini P, Ferrari A, Catricala C. Malignant melanoma and pregnancy. Melanoma Res 2006;16:497–500.

46. Lens MB, Rosdahl I, Ahlbom A, et al. Effect of pregnancy on survival in women with cutaneous malignant melanoma. J Clin Oncol 2004;22:4369–75.

47. Flowers JL, Seigler HF, McCarty KS, Sr., Konrath J, McCarty KS, Jr. Absence of estrogen receptor in human melanoma as evaluated by a monoclonal antiestrogen receptor antibody. Arch Dermatol 1987; 123:764–5.

48. Cohen C, DeRose PB, Campbell WG, Schlosnagle DC, Sgoutas D. Estrogen receptor status in malignant melanoma. Am J Dermatopathol 1990;12:562–4.

49. Duncan LM, Travers RL, Koerner FC, Mihm MC, Jr., Sober AJ. Estrogen and progesterone receptor analysis in pregnancy-associated melanoma: absence of immunohistochemically detectable hormone receptors. Hum Pathol 1994;25:36–41.

50. Lecavalier MA, From L, Gaid N. Absence of estrogen receptors in dysplastic nevi and malignant melanoma. J Am Acad Dermatol 1990;23:242–6.

51. Piantelli M, Maggiano N, Ricci R, et al. Tamoxifen and quercetin interact with type II estrogen binding sites and inhibit the growth of human melanoma cells. J Invest Dermatol 1995;105:248–53.

52. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 1996;392:49–53.

53. Schmidt AN, Nanney LB, Boyd AS, King LE, Jr., Ellis DL. Oestrogen receptor-beta expression in melanocytic lesions. Exp Dermatol 2006;15:971–80.

54. Lens MB, Reiman T, Husain AF. Use of tamoxifen in the treatment of malignant melanoma. Cancer 2003; 98:1355–61.

55. Duong BN, Elliott S, Frigo DE, et al. AKT regulation of estrogen receptor beta transcriptional activity in breast cancer. Cancer Res 2006;66:8373–81.

56. Esslimani-Sahla M, Simony-Lafontaine J, Kramar A, et al. Estrogen receptor beta (ER beta) level but not its ER beta cx variant helps to predict tamoxifen resistance in breast cancer. Clin Cancer Res 2004;10: 5769–76.

57. Speirs V, Malone C, Walton DS, Kerin MJ, Atkin SL. Increased expression of estrogen receptor beta mRNA in tamoxifen-resistant breast cancer patients. Cancer Res 1999;59:5421–4.

58. Boersma BJ, Howe TM, Goodman JE, et al. Association of breast cancer outcome with status of p53 and MDM2 SNP309. J Natl Cancer Inst 2006;98:911–9.

59. Copson ER, White HE, Blaydes JP, Robinson DO, Johnson PW, Eccles DM. Influence of the MDM2 single nucleotide polymorphism SNP309 on tumour development in BRCA1 mutation carriers. BMC Cancer 2006;6:80.

60. Ohmiya N, Taguchi A, Mabuchi N, et al. MDM2 promoter polymorphism is associated with both an increased susceptibility to gastric carcinoma and poor prognosis. J Clin Oncol 2006;24:4434–40.

61. Walsh CS, Miller CW, Karlan BY, Koeffler HP. Association between a functional single nucleotide polymorphism in the MDM2 gene and sporadic endometrial cancer risk. Gynecol Oncol 2007;104:660–4.

62. Alazzouzi H, Suriano G, Guerra A, et al. Tumour selection advantage of non-dominant negative P53 mutations in homozygotic MDM2–309 colorectal cancer cells. J Med Genet 2007;44:75–80.

63. Wilkening S, Hemminki K, Rudnai P, et al. No association between MDM2 SNP309 promoter polymorphism and basal cell carcinoma of the skin. Br J Dermatol 2007;157:375–7.

64. Atwal GS, Bond GL, Metsuyanim S, et al. Haplotype structure and selection of the MDM2 oncogene in humans. Proc Natl Acad Sci U S A 2007;104:4524–9.

65. Nan H, Qureshi AA, Hunter DJ, Han J. A functional SNP in the MDM2 promoter, pigmentary phenotypes, and risk of skin cancer. Cancer Causes Control 2009; 20:171–9. Epub 2008 Sep 24.